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ORIGINAL ARTICLE

Antimicrobial resistance in diarrheagenic *Escherichia coli* from ready-to-eat foods

Cíntia Matos Lima[1] · Ingrid Evelyn Gomes Lima Souza[1] · Taila dos Santos Alves[2] · Clícia Capibaribe Leite[1] · Norma Suely Evangelista-Barreto[3] · Rogeria Comastri de Castro Almeida[4]

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Abstract Certain subgroups of *Escherichia coli* have congenital or acquired virulence properties that allow them to cause a wide spectrum of disease. The aim of this study was to investigate the occurrence of diarrheagenic *E. coli* strains in ready-to-eat (RTE) foods produced in institutional, commercial and hotel restaurants in Salvador, Brazil. The presence of virulent isolates and antimicrobial resistance were evaluated. Four hundred forty-six samples were collected and grouped into cereals and vegetables, meat-based preparations, cooked salads, raw salads, garnishes, soups and sauces, desserts and juices. *E. coli* were detected using the most probable number method, the presence of virulence factors in isolates was determined by polymerase chain reaction (PCR) assays, and antibiotic resistance was analyzed using the disc diffusion method. In total, 15 isolates (3.1%) of *E. coli* were recovered; raw salads had the highest detection rate, 1.4%, followed by cooked salads, 0.8%; meat-based preparations, 0.4%; and cereals and vegetables, 0.4%. PCR assays showed that none of the isolates had the virulence genes *cnf1, cnf2, eae, sta, lt1, stx1, stx2* or *cdtB*. The isolates showed resistance to nine antibiotics of the 15 tested, and the highest levels of resistance were found for sulfamethoxazole/trimethoprim, tetracycline, ampicillin, and chloramphenicol (13.3% of isolates for each antibiotic). One isolate from cooked salad had plasmid-mediated multidrug resistance to tetracycline, trimethoprim/sulfamethoxazole, ampicillin and chloramphenicol. These results suggest that RTE foods, especially raw salads, can be reservoirs of *E. coli* and facilitate the spread of antibiotic resistance genes to the gastrointestinal microbiota of humans.

Keywords *Escherichia coli* · RTE foods · Virulence · Antibiotic · Resistance

✉ Rogeria Comastri de Castro Almeida
rogeriac@ufba.br

[1] Pharmacy Faculty, Federal University of Bahia, Rua Barão de Geremoabo, s/n, Ondina, Salvador, BA Cep: 40170-290, Brazil

[2] Genetic, Evolution and Bioagents Department, Biology Institute, Campinas University, Barão Geraldo, Campinas, SP Cep: 13083-862, Brazil

[3] Center for Research on Fisheries and Aquaculture (NEPA), Federal University of Bahia Recôncavo, Cruz das Almas, BA Cep: 44380-000, Brazil

[4] Nutrition School, Federal University of Bahia, Av. Araújo Pinho, No. 32, Canela, Salvador, BA Cep: 40110-160, Brazil

Introduction

The occurrence and consequences of food-borne diseases have been underestimated because they are not usually reported, and establishing causal links between food contamination and disease or death is difficult (WHO 2015).

Escherichia coli is one of the most versatile and well-studied microorganisms worldwide. *Escherichia coli* is typically present in the gastrointestinal tract of humans and warm-blooded animals (Jiang et al. 2014); however, the microorganism is also able to grow in several extra-intestinal environments (APHA 2001).

The presence of *E. coli* in food may indicate fecal contamination, the use of contaminated raw materials, unsatisfactory hygienic conditions in the food preparation environment and the presence of other enteric pathogens (Jiang et al. 2014).

Although *E. coli* is inactivated by thermal treatments in processed food, post-processing cross-contamination from

equipment and the environment may occur due to the pathogen's persistence (Yang et al. 2016).

Although most strains are nonpathogenic, certain subgroups of *E. coli* have congenital or acquired virulence factors that provide a greater ability to adapt to new environments, allowing them to cause a wide spectrum of disease (Kaper et al. 2004).

In particular, diarrheagenic *E. coli* is the main subgroup involved in gastrointestinal diseases caused by the ingestion of contaminated ready-to-eat (RTE) foods (Li et al. 2016).

In the United States, data from the Centers for Disease Control and Prevention (CDC) estimate that from 2000 to 2008, *E. coli* was involved in 203,000 cases of food-borne diseases, causing 21 deaths (CDC 2012). These data indicate that the lack of control in food processing steps or the lack of adoption of good manufacturing practices (GMPs) is worrisome with regard to RTE foods. In Brazil, *E. coli* was identified as the etiologic agent involved in 719 cases of food-borne diseases from 2000 to 2015. It was the third most prevalent microorganism in these types of outbreaks, preceded by only *Salmonella* spp. and *Staphylococcus aureus* (Brasil 2016).

The pathogenesis of *E. coli* is associated with virulence factors responsible for colonization and infection in humans. Each *E. coli* subgroup has specific genes responsible for coding virulence factors that interfere with host physiology. Among the most important genes, *stx* has been associated with *E. coli* strains producing Shiga toxin (STEC). STEC are one of the most important strains, mainly due to their ability to cause an array of diseases (Peresi et al. 2016; Li et al. 2016). The genes *st* (enterotoxin heat-stable) and *lt* (enterotoxin heat-labile) have been associated with enterotoxigenic *E. coli* (ETEC), the most common cause of childhood diarrhea (Canizalez-Roman et al. 2013). Enteropathogenic *E. coli* (EPEC) strains contain the *eae* and *bfp*A genes that encode the proteins intimin and bundle-forming pilus (BFP), respectively (Canizalez-Roman et al. 2013).

To reduce morbidity and mortality as well as the economic impact associated with these bacterial infections, antibiotics have been used for many years in human and veterinary medicine. However, *E. coli* has shown increased resistance to one or more antibiotics (Silva and Mendonça 2012), a fact that has generated concern for public health.

The high prevalence of resistant strains is related to the indiscriminate and increasingly high antibiotic use. In veterinary medicine, for example, the use of these drugs at low doses for extended durations to feed animals can causes the selection and spread of antibiotic resistance to other bacteria in the food chain (Ryu et al. 2012; Silva and Mendonça 2012).

The present study investigated the occurrence of diarrheagenic *E. coli*, the presence of virulent isolates and antimicrobial resistance in RTE foods collected in Salvador (Brazil).

Materials and methods

Assessment of *Escherichia coli* in RTE foods

From September 2014 to February 2015, a total of 486 samples of RTE foods were collected in commercial (73), institutional (239), and hotel restaurants (174) in Salvador, Brazil. These restaurants were located in different regions of the city and were visited at least two times in the same month for sample collection.

The sample types included 101 cereals and vegetables (including rice, pasta and grains), 161 meat-based preparations (including bovine, swine, poultry, and seafood), 45 cooked salads (including cooked vegetables occasionally with added mayonnaise, fruits, grains and yogurt), 90 raw salads (including raw vegetables and fruits), 30 garnishes (including cooked vegetables with added milk, cheese, eggs, grains or oilseeds), 20 soups and sauces (made from cooked vegetables with added meat, grains, mustard and mayonnaise), 25 desserts (including frozen pies, cakes, musses and puddings), and 14 juices (made from fruits and fruit pulps, water and sugar).

For the detection of thermotolerant coliforms and *E. coli*, 250 g of food was aseptically collected in the restaurants and transported to the laboratory for analysis. Twenty-five grams of each sample was added to 225 ml of buffer peptone water (BPW) aseptically and homogenized in a Stomacher (240 bpm; Bagmixer® 400, São Paulo, SP, Brazil) for 2 min. The most probable number (MPN) methodology was performed using a sequence of three tubes per sample dilution according to Jeffrey et al. (2001). Positive tubes from the EC broth were streaked on eosin-methylene blue (EMB, Acumedia, São Paulo, SP, Brazil) agar, and for the identification of *E. coli*, at least two colonies (black center and metallic luster) were selected from each EMB plate. Biochemical confirmation of the isolates was performed using the IMViC tests (Indole, Methyl Red, Voges–Proskauer, and Citrate tests) (Jeffrey et al. 2001). *Escherichia coli* ATCC 25922 (personal collection) was used as positive control in all assays.

Identification of the *Escherichia coli* serogroups

The identification of the serogroups of the *E. coli* isolates was performed using the agglutination test, following recommendations of the manufacturer (Probac do Brasil®, São Paulo, SP, Brazil). Enteroinvasive *E. coli* (EIEC), polyvalent antisera *E. coli* A (*E. coli* 028ac, 029, 0136, O144 and 0152), and polyvalent *E. coli* B (*E. coli* 0112ac,

0124, 0143, 0164 and O167) were used. The identification of enterohemorrhagic *Escherichia coli* (EHEC) was performed with *E. coli* O157 antiserum, and enteropathogenic *E. coli* (EPEC) was identified with classic polyvalent antiserum A (*E. coli* 026, 055, 0111 and 0119), polyvalent antiserum B (*E. coli* 0114, 0125, 0142 and 0158) and polyvalent antiserum C (*E. coli* 086, 0126, 0127 and 0128) (Orskov et al. 1984).

Detection of virulence genes in the *Escherichia coli* isolated

The presence of virulence genes in the *E. coli* isolates was assessed by PCR detection using primers described in Table 1.

For DNA extraction, one loop of bacterial growth obtained by incubation of the isolates at 37 °C overnight on tryptic soy agar (TSA, Difco, Detroit, MI, USA) was suspended in 100 µl of sterile ultra-high quality (UHQ) water, boiled for 10 min and then centrifuged at 10,000×*g* (Sorvall® Centrifuge MC12, São Paulo, SP, Brazil) for 3 min. The supernatant was used as the template for PCR assays (Siqueira et al. 2009).

The PCR assays were performed in a final volume of 30 µl, using 3 µl of 10 × PCR buffer, 2.5 mM of $MgCl_2$, 400 µM dNTPs, 1.5 U of Taq DNA polymerase, sterile UHQ water (qsp), 7 µl of template DNA and 1 µl of each primer (Siqueira et al. 2009). The concentration of the primers, annealing temperatures, predicted sizes of the amplified products (amplicons), and positive control are shown in Table 1.

The amplifications were executed in a thermocycler (Techne TC-312, Burlington, NJ, USA) as follows: an initial denaturation at 94 °C for 5 min; 30 cycles of 94 °C for 1 min, the annealing temperature for 1 min, and 72 °C for 2 min; and a final extension at 72 °C for 7 min. *E. coli* K12C600 was used as a negative control (Siqueira et al. 2009).

The amplified fragments were subjected to electrophoresis on a 2% agarose gel stained with ethidium bromide solution (1 µg/ml) (Sigma, São Paulo, SP, Brazil) and were visualized using a UV transilluminator coupled with a digital gel imaging system (Kodak EDAS 290) (Siqueira et al. 2009).

Susceptibility of *Escherichia coli* isolates to antimicrobials

Escherichia coli isolates were submitted for susceptibility testing toward 15 antimicrobials using the disk diffusion method (CLSI 2013). Cells were grown at 37 °C for 18 h in tryptic soy broth (TSB, Difco, Detroit, MI, USA) to reach stationary phase and then adjusted to 0.5 points on the McFarland scale (ca. 10^8 cfu) in a saline physiologic

Table 1 Primers, amplicon sizes and amplification conditions for the PCR assays

Gene target	Sequence (5′–3′)	Concentration (ng/µl)	Annealing temperature (°C)	Amplicon size (bp)	Control strain	References
cnf1	F = GAACTTATTAAGGATAG R = CATTATTTATAACGCTG	90	43	543	J96	Blanco et al. (1996)
cnf2	F = AATCTAATTAAAGAGAAC R = CATGCTTTGTATATCTA	90	43	543	UEC054 (B62)	Blanco et al. (1996)
Eae	F = GACCCGGCACAAGCATAAGG R = CCACCTGCAGCAACAAGAGC	60	63	384	UEC017 (O157:H7) H30 (O26:H11)	Yu and Kaper (1992)
Sta	F = TTAATAGCACCCGGTACAAGCAGG R = CTTGACTCTTCAAAAGAGAAAATTAC	60	50	176	UEC019 (FV811)	Olsvik and Strockbine (1993)
lt1	F = GGCGACAGATTATACCGTGC R = CCGAATTCTGTTATATATGTC	90	55	696	UEC021 (FV814)	Schultsz et al. (1994)
stx1	F = AAGTTGCAGCTCTCTTTGAATA R = TGCAAACAAATTATCCCCTGAG	90	60	364	H30	Ojeniyi et al. (1994)
stx2	F = GGGCAGTTATTTTGCTGTGGA R = GTATCTGCCTGAAGCGTAA	90	60	515	UEC021 (FV814) J29	Ojeniyi et al. (1994)
cdtB	F = GAGTTATTCCTTCCCCAGGC R = CAAAGGCATCAACAGCAGAA	0	58	108	CLDT (7)	Silva and Leite (2002)

solution (prepared in the laboratory as recommended by the APHA 2001). The cell suspensions were inoculated on Mueller–Hinton (MH) agar (HiMedia, São Paulo, SP, Brazil) using a sterile swab (Laborclin, São Paulo, SP, Brazil). The susceptibility of *E. coli* isolates to each antibiotic (Laborclin, São Paulo, SP, Brazil) was determined at concentrations corresponding to breakpoints indicated by the Clinical and Laboratory Standards Institute (CLSI 2013): 10 µg ampicillin (AMP), 20/10 µg amoxicillin/clavulanic acid (AMC), 30 µg cephalothin (CF), 30 µg cefotaxime (CTX), 30 µg cefoxitin (CFX), 30 µg ceftazidime (CAZ), 10 µg gentamicin (GEN), 30 µg amikacin (AMI), 30 µg kanamycin (KAN), 10 µg streptomycin (STR), 30 µg tetracycline (TET), 5 µg ciprofloxacin (CIP), 30 µg nalidixic acid (NAL), 1.25 µg/23.75 µg trimethoprim/sulfamethoxazole (STX), and 30 µg chloramphenicol (CL). The length of the inhibition halo was measured after 16–18 h at 35 °C. *Escherichia coli* ATCC 25922 were included as quality control in all assays. Antibiotic susceptibility was classified as sensitive, intermediate or resistant on the basis of the CLSI breakpoints.

The antibiotics were chosen on the basis of their importance in human therapy or for treating animal infections caused by Gram-negative bacteria in the Enterobacteriaceae family.

For each isolate of *E. coli*, the multiple antibiotic resistance index (MAR) was calculated by considering the number of antimicrobial agents to which the isolate was resistant divided by the total of antimicrobial agents tested (Krumperman 1983).

Detection of resistance mediated by R-plasmids

The presence of an R-plasmid in the antibiotic-resistant isolates was investigated. Acridine orange (Sigma-Aldrich, São Paulo, SP, Brazil) was used as a curing reagent at concentrations of 100 µg/ml and 200 µg/ml. First, the resistant isolates were incubated in nutrient broth (Himedia, São Paulo, SP, Brazil) at 37 °C for 24 h, and then, 200-µl aliquots of the culture were added to tubes containing Luria–Bertani broth (prepared in the laboratory as recommended by the APHA (2001)) plus acridine orange, followed by further incubation at 37 °C for 24 h. Subsequently, the isolates were subjected again to susceptibility testing with the previously tested antimicrobials to check for changes in their resistance profiles (Molina-Aja et al. 2002).

Statistical analysis

To test the existence of an association between qualitative variables, the Chi square test was performed using R software (version 3.2.1). The null hypothesis (H_0) that there is no association between the variables was tested. The results were considered significant when the p value was less than the significance level of $\alpha = 0.05$.

Results and discussion

Escherichia coli is the most widespread indicator of fecal contamination in food, and its presence usually correlates with inadequate hygiene, inappropriate food handling, cross contamination and/or inappropriate food storage conditions (Whyte et al. 2004).

In the present study, raw salads, cooked salads and juices showed the highest rate of samples with MPNs of thermotolerant coliforms unfit for human consumption (Brasil 2001).

Regarding the occurrence of *E. coli*, 15 (3.0%) samples of the RTE foods were found to be contaminated. Raw salads had the highest rate of contamination, 1.4% (n = 7), followed by cooked salads, 0.8% (n = 4); meat-based preparations, 0.4% (n = 2); and cereals and legumes, 0.4% (n = 2) (Table 2).

No association was found between the site of sampling (restaurants) and the type of RTE food evaluated. Nevertheless, the type of RTE food was associated with the presence of *E. coli* ($p = 0.01897$).

In Brazil, 1.2% of reported food-borne diseases from 2000 to 2015 were related to the consumption of vegetables (Brasil 2016).

Serological tests identified five isolates as enteropathogenic *E. coli* (EPEC), three as enteroinvasive (EIEC), and one as enterohemorrhagic (EHEC); six isolates were negative for the agglutination test. Among the enteropathogenic serotypes isolated, two came from cooked salad, two from raw salad, and one from cereals and legumes. Among the isolates identified as enteroinvasive *E. coli*, two came from meat-based preparations and one from a cooked salad. The enterohemorrhagic isolate came from raw salad.

PCR assays showed that none of the *E. coli* isolates possessed the virulence genes *cnf1, cnf2, eae*, *sta, lt1, stx1, stx2* or *cdtB* that were investigated.

Recent studies developed by some researchers report isolation rates of *E. coli* in RTE foods between 1.0 and 3.0% (Ryu et al. 2012; Canizalez-Roman et al. 2013; Yang et al. 2016), similar to the results found in the present study. In addition, studies with raw vegetables, the food type with a higher rate of contamination by *E. coli* in this study, have demonstrated that the number of gastroenteritis outbreaks related to the consumption of raw leafy vegetables is significant and increases every year (Taban and Halkman 2011; Olaimat and Holley 2012).

The high MPN of thermotolerant coliforms detected in raw salads investigated in this study may be related to the

Table 2 Occurrence of thermotolerant coliforms and *E. coli* in RTE foods from commercial, institutional and hotel restaurants in Salvador, Brazil

RTE foods	Restaurants/samples n (%)	Positive samples	
		Thermotolerant coliforms n (%)	*Escherichia coli* n (%)
Cereals and legumes	Hotel/29 (6.0)	1 (0.7)	0 (0.0)
	Commercial/17 (3.5)	5 (3.7)	1 (6.7)
	Institutional/55 (11.3)	4 (3.0)	1 (6.7)
Total	101 (20.8)	10 (2.1)	2 (0.4)
Meat-based	Hotel/55 (11.3)	3 (2.2)	0 (0.0)
	Commercial/26 (5.4)	9 (6.6)	2 (13.3)
	Institutional/80 (16.5)	9 (6.6)	0 (0.0)
Total	161 (33.1)	21 (4.3)	2 (0.4)
Garnish	Hotel/14 (2.9)	0 (0.0)	0 (0.0)
	Commercial/3 (0.6)	0 (0.0)	0 (0.0)
	Institutional/13 (2.7)	1 (0.7)	0 (0.0)
Total	30 (6.2)	1 (0.2)	0 (0.0)
Cooked salad	Hotel/19 (3.9)	3 (2.2)	0 (0.0)
	Commercial 6 (1.2)	6 (4.4)	3 (20.0)
	Institutional/20 (4.1)	16 (11.8)	1 (6.7)
Total	45 (9.3)	25 (5.1)	4 (0.8)
Raw salad	Hotel/26 (5.3)	16 (11.8)	3 (20.0)
	Commercial/15 (3.1)	9 (6.6)	1 (6.7)
	Institutional/49 (10.1)	46 (33.8)	3 (20.0)
Total	90 (18.5)	71 (14.6)	7 (1.4)
Desserts	Hotel/16 (3.3)	3 (2.2)	0 (0.0)
	Commercial/3 (0.6)	0 (0.0)	0 (0.0)
	Institutional/6 (1.2)	0 (0.0)	0 (0.0)
Total	25 (5.1)	3 (0.6)	0 (0.0)
Soups and sauces	Hotel/10 (2.1)	0 (0.0)	0 (0.0)
	Commercial/1 (0.2)	0 (0.0)	0 (0.0)
	Institutional/9 (1.9)	2 (1.5)	0 (0.0)
Total	20 (4.1)	2 (0.4)	0 (0.0)
Juices	Hotel/5 (1.0)	1 (0.7)	0 (0.0)
	Commercial/2 (0.4)	0 (0.0)	0 (0.0)
	Institutional/7 (1.4)	2 (1.5)	0 (0.0)
Total	14 (2.9)	3 (0.6)	0 (0.0)
Total	486 (100.0)	136 (27.9)	15 (3.0)

exposure of plants to contaminants during irrigation, harvesting, transport, processing and sale (Gómez-Aldapa et al. 2013).

With regard to cooked foods in general, control of the time/temperature binomial is sufficient to eliminate the biological agents that compromise food safety. Thus, the contamination of cooked foods by diarrheagenic *E. coli*, as found in this study, is indicative of inadequate cooking, inappropriate handling or cross contamination (Bautista-De Léon et al. 2013; Sospedra et al. 2013). Therefore, non-compliance with GMPs is the main cause of food-borne illnesses that are transmitted by food infected with the EHEC, EPEC and EIEC types of *E. coli* (Newell et al. 2010).

According to Min et al. (2017), conventional post-harvest washing and sanitizing treatments are not completely effective for the decontamination of vegetables and fresh fruits; thus, alternative methods or new technologies are necessary to prevent contamination by food-borne pathogens in these products.

The failure to detect the genes *cnf2, sta, lt1, stx1* and *stx2* in this study may be related to the loss of genetic material in mobile genetic elements (plasmids and bacteriophages) (Kaper et al. 2004). These virulence factors,

when located in mobile genetic elements, are an epidemiological concern since they may facilitate the spread of virulent bacteria (Silva and Mendonça 2012).

The susceptibility profiles of the 15 *E. coli* isolates to the 15 antibiotics tested is shown in Table 3. The isolates showed resistance to nine (60%) of the antibiotics tested; specifically, 13.3% (n = 2) were resistant to the group of sulfonamides (trimethoprim/sulfamethoxazole) and tetracycline, 13.3% (n = 2) to the group of beta-lactams (ampicillin, n = 2; cephalothin, n = 1; cefotaxime, n = 1; and cefoxitin, n = 1), 13.3% (n = 2) to chloramphenicol, 6.7% (n = 1) to the group of quinolones (nalidixic acid, n = 1), and 6.7% were resistant to aminoglycosides (streptomycin, n = 1).

Among the isolates, two (13.3%) showed resistance to at least four antibiotics. The isolates from a cereal and legume samples presented a MAR equal to 0.53 or 53% (resistance to eight antibiotics: cefotaxime, ampicillin, cephalothin, tetracycline, trimethoprim/sulfamethoxazole, nalidixic acid, streptomycin, and chloramphenicol), and the MAR for the isolate from cooked salad was equal to 0.27 or 27% (resistance to four antibiotics: tetracycline, trimethoprim/sulfamethoxazole, ampicillin, and chloramphenicol).

According to Newell et al. (2010), the use of antimicrobials for human therapy, animal health or plant health purposes can select for the emergence of resistance and promote the dissemination of resistant bacteria and resistance genes. Furthermore, commensal *E. coli* can be a source of resistance genes for pathogenic *E. coli* strains. Recent studies involving RTE foods performed by Canizalez-Roman et al. (2013) identified *E. coli* strains resistant to tetracycline (34%), cefotaxime (30%) and ampicillin (29%). Moreover, the authors noted that 60% of the EPEC strains were resistant to cefotaxime (34%) and tetracycline (23%). Campos et al. (2013) also related that EPEC strains were resistant to tetracycline, streptomycin, and sulfamethoxazole/trimethoprim, similar results to those of the present study.

In this study, we found that only the EPEC isolates were multidrug-resistant, and one of them, isolated from a cooked salad prepared by a commercial restaurant, has a multidrug-resistance plasmid that mediates resistance to tetracycline, trimethoprim/sulfamethoxazole, ampicillin, and chloramphenicol. The results from work performed by Barros et al. (2012) indicated that 94% of *E. coli* strains from chicken samples were multidrug-resistant to the antibiotics tested, with MARs higher than those found in the present study. The same authors demonstrated the presence of plasmids in 80% of the multidrug-resistant strains. Although food of animal origin is the main food source that shows antimicrobial-resistant bacteria/genes, contamination during the handling and preparation of food of plant origin, such as salads, is of concern (Campos et al. 2013).

In general, the identification of pathogenic multidrug-resistant *E. coli* strains in RTE foods represents a risk of infection for humans. In addition, the presence of plasmids in the tested antimicrobial-resistant isolates indicates the possibility of these genes being spread to other pathogens and commensal residents in the human gastrointestinal tract, leading to difficulties in the selection and use of appropriate therapeutic treatments.

Table 3 Susceptibility of the *E. coli* isolates to antibiotics

Antibiotics	Isolates *n* (%)		
	Resistant	Intermediately resistant	Susceptible
Ampicillin	2 (13.3)	ND	13 (86.7)
Amikacin	ND	1 (6.7)	14 (93.3)
Cephalothin	1 (6.7)	1 (6.7)	13 (86.6)
Cefotaxime	1 (6.7)	ND	14 (93.3)
Cefoxitin	1 (6.7)	ND	14 (93.3)
Ceftazidime	ND	ND	15 (100.0)
Gentamicin	ND	ND	15 (100.0)
Amikacin	ND	ND	15 (100.0)
Kanamycin	ND	ND	15 (100.0)
Streptomycin	1 (6.7)	3 (20.0)	11 (73.3)
Tetracycline	2 (13.3)	ND	13 (86.7)
Ciprofloxacin	ND	ND	15 (100.0)
Nalidixic acid	1 (6.7)	1 (6.7)	13 (86.6)
Trimethoprim/sulfamethoxazole	2 (13.3)	ND	13 (86.7)
Chloramphenicol	2 (13.3)	ND	13 (86.7)

ND Not detected

Conclusion

Results obtained in this study indicated that RTE foods, particularly raw salads, present health risks to consumers due to contamination by multidrug-resistant EPEC. This risk is understood as the capability of the pathogen to transfer multi-resistance genes to other pathogenic microorganisms and/or commensal residents of the human gut, leading to difficulties in the selection and use of appropriate therapeutic treatments.

Monitoring the antimicrobial resistance and evolution of *E. coli* pathotypes is very important. Additionally, proper hygiene practices and the adoption of GMPs are needed to constrain strain dissemination throughout the entire food chain.

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Compliance with ethical standards

Conflict of interest The authors declares that they have no conflict of interest.

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